Denaturing Cell Extraction Buffer

Catalog Number FNN0091

Pub. No. MAN0016093 **Rev.** 2.0 (30)

Product Description

Cat. No.	FNN0091
Lot No.	See product label.
Quantity	100 mL
Description	Denaturing Cell Extraction Buffer is suitable for use in ELISA and western blotting. This can be used for Invitrogen™ phosphoELISAs (i.e., ERK, JNK, and MEK pELISAs) that require Sample Treatment (Invitrogen™ Cat. No. ST001) or boiling. After lysing cells with Denaturing Cell Extraction Buffer, lysates will not have to be sample-treated or boiled.
Buffer Formulation	Proprietary **Note:** This Denaturing Cell Extraction buffer must be supplemented with 1 mM PMSF and protease inhibitor cocktail (i.e, Sigma-Aldrich™ Cat. No. P-2714) just prior to use. • For the PMSF addition, we recommend making a 0.3 M stock in DMSO, and adding sufficient volume for a final concentration of 1 mM (i.e., 17 µL per 5 mL extraction buffer). PMSF is very unstable and must be added just prior to use, even if added previously. • For the protease inhibitor cocktail addition, we recommend Sigma-Aldrich™ Cat. No. P-2714, reconstituted according to the manufacturer's instructions, added at 100 µL per 1 mL Denaturing Cell Extraction Buffer. The stability of protease inhibitor-supplemented cell extraction buffer is 24 hours at 4°C.
Instructions	This protocol has been successfully applied to several cell lines. Some optimization may be required for each specific application. 1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent). 2. Wash cells twice with cold PBS. 3. Remove and discard the supernatant and collect the cell pellet. 4. Lyse the cell pellet in Denaturing Cell Extraction Buffer for 30 minutes, on ice, with vortexing at 10-minute intervals. The volume of extraction buffer depends on the cell number and expression of target protein and level of phosphorylation. A suitable starting concentration is 10^7 cells per mL of extraction buffer. 5. Transfer the extract to microcentrifuge tubes and centrifuge at 13,000 rpm for 15 minutes at 4°C. 6. Aliquot the clear lysate into clean microcentrifuge tubes. These samples are ready to assay. Lysates can be stored at -80°C. Avoid multiple freeze/thaw cycles.
Storage	Store at ≤-20°C. Thaw this buffer on ice. This buffer is stable for 2-3 weeks at 2-8°C or for up to 1 year when apportioned into working aliquots and stored at ≤-20°C.

Manufacturer: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

ThermoFisher SCIENTIFIC